The Influence of Membrane Composition on the Kinetics of Influenza Virus Fusion Measured using a Single Particle Approach

van der Borg, Guus; Braddock, Scarlett; Blijleven, Jelle S.; van Oijen, Antoine M.; Roos, Wouter H.

Published in:
Biophysical Journal

DOI:
10.1016/j.bpj.2016.11.475

Virus pseudotyping is a useful and safe technique for studying entry of emerging strains of influenza virus. However, few studies have compared different reassortant combinations in pseudoparticle systems, or compared entry kinetics of native viruses and their pseudotyped analogs. Here, vesicular stomatitis virus (VSV)-based pseudovirions displaying distinct influenza virus envelope proteins were tested for fusion activity. We produced VSV pseudotypes containing the prototypical X-31 (H3) HA, either alone or with strain-matched or mismatched N2 NAs. We performed single-particle fusion assays using total internal reflection fluorescence microscopy to compare hemifusion kinetics among these pairings. Results illustrate that matching pseudoparticles behaved very similarly to native virus. Pseudoparticles harboring mismatched HA-NA pairings fuse at significantly slower rates than native virus, and NA-lacking pseudoparticles exhibiting the slowest fusion rates. Relative viral membrane HA density of matching pseudoparticles was higher than in mismatching or NA-lacking pseudoparticles. An equivalent trend of HA expression level on cell membranes of HA/NA co-transfected cells was observed and intracellular trafficking of HA was affected by NA co-expression. Overall, we show that specific influenza HA-NA combinations can profoundly affect the critical role played by HA during entry, which may factor into viral fitness and the emergence of new pandemic influenza viruses.

405-Pos Board B170

SERINC5 Inhibits HIV Fusion through Inactivation of Env Glycoproteins and Interference with Productive Refolding of Env

Chetan Sood[1], Mariana Marin[1], Ajit Chande[2], Alexa L. Mattheyses[3], Khalid Salaita[4], Massimo Pizzato[5], **Gregory Melikian**[1].
[1]Pediatrics, Emory University, Atlanta, GA, USA, [2]University of Trento, Trento, Italy, [3]Cell biology, Emory University, Atlanta, GA, USA, [4]Chemistry, Emory University, Atlanta, GA, USA, [5]University, Trento, Italy.

The multispan membrane proteins, SERINC3 and SERINC5, have been recently shown to incorporate into HIV-1 particles and compromise their ability to fuse with target cells – an effect that is antagonized by the viral accessary protein Nef. Env glycoproteins from different HIV-1 strains exhibit variable levels of sensitivity to SERINC-mediated restriction. The mechanism by which SERINCs interfere with HIV-1 fusion remains unclear. Here, we show by real-time single particle imaging that incorporation of SERINC5 into virions in the absence of Nef inhibits the formation of small fusion pores between viruses and cells. This effect was not related to the SERINC5's ability to oligomerize in the membrane or target the virus to degradation in lysosomes. Strikingly, we found that SERINC5 promotes spontaneous inactivation of sensitive, but not resistant Env glycoproteins, and enhances the exposure of the conserved gp41 domains by delaying the HIV-1 fusion reaction. Super-resolution imaging revealed that SERINC5 also interferes with the formation of Env clusters on mature virions, a step that is thought to be required for efficient HIV-1 fusion. These results show that SERINC5 restricts HIV-1 fusion at a step prior to small pore formation by selectively inactivating sensitive Env glycoproteins and interfering with the function of the remaining active Env, likely by preventing the formation of large Env clusters and slowing down Env refolding. This work was partially supported by the NIH R01 grant GM054787 to G.B.M.

406-Pos Board B171

SERINC Inhibits HIV-1 Env Induced Membrane Fusion and Slows Fusion Pore Enlargement

Ruben M. Markosyan[1], Shan-Lu Liu[2], Fred S. Cohen[1].
[1]Rush University Medical Center, Chicago, IL, USA, [2]Department of Molecular Microbiology and Immunology, University of Missouri School of Medicine, Columbia, MO, USA.

The SERINC family of proteins are integral membrane proteins that regulate the incorporation of serine into phospholipids, to create PS, and into sphingolipids. It has recently been shown that two members of the family, SERINC3 and SERINC5, inhibit HIV infectivity. Using a cell-cell fusion system to determine the extent to which inhibition of infectivity is due to reduced fusion, we found that the presence of SERINC3 or SERINC5 in either effector or target cells slows the kinetics and reduces the extent of fusion induced by HIV-1 Env. These two incorporators of serine greatly retard fusion pore enlargement, as determined by the rate of aqueous dye transfer once a pore forms. Nef is an auxiliary protein of HIV that is well-known to enhance HIV infectivity. The presence of SERINC5 and Nef in effector cells leads to the same extent of fusion induced by expression of Env alone, showing that Nef eliminates the reduction of fusion caused by SERINC. (R01 GM 101 539).

407-Pos Board B172

Probing Induced Structural Changes in Biomimetic Bacterial Cell Membrane Interactions with Divalent Cations

Allison Whited, Alexander Johs, John Katsaras, Robert Standaert, Aaron Jubb.
Oak Ridge National Lab, Knxoville, TN, USA.

Biological membranes, formed primarily by the self-assembly of complex mixtures of phospholipids, provide a structured scaffold for compartmentalization and structural processes in living cells. The specific physical properties of phospholipid species present in a given membrane play a key role in mediating these processes. Phosphatidylethanolamine (PE), a zwitterionic lipid present in bacterial, yeast, and mammalian cell membranes, is exceptional. In addition to undergoing the standard lipid polymorphic transition between the gel and liquid-crystalline phase, it can also assume an unusual polymorphic state, the inverse hexagonal phase (H_{II}). Divalent cations are among the factors that drive the formation of the H_{II} phase, wherein the lipid molecules form stacked tubular structures by burying the hydrophilic head groups and exposing the hydrophobic tails to the bulk solvent. Most biological membranes contain a lipid species capable of forming the H_{II} state suggesting that such lipid polymorphic structural states play an important role in structural biological processes such as membrane fusion. In this study, the interactions between Mg^{2+} and biomimetic bacterial cell membranes composed of PE and phosphatidylglycerol (PG) were probed using differential scanning calorimetry (DSC), small-angle x-ray scattering (SAXS), and fluorescence spectroscopy. The lipid phase transitions were examined at varying ratios of PE to PG and upon exposure to physiologically relevant concentrations of Mg^{2+}. An understanding of these basic interactions enhances our understanding of membrane dynamics and how membrane-mediated structural changes may occur *in vivo*.

408-Pos Board B173

Role of *trans* to *cis* Transition in Viral Fusion Pore Dilation

Brett E. Alcott[1], Zhenyong Wu[2,3], Josie Bircher[4], Erdem Karatekin[2,3], Ben O' Shaughnessy[5].
[1]Biochemistry and Molecular Biophysics, Columbia University, New York, NY, USA, [2]Cellular and Molecular Physiology, Yale University, New Haven, CT, USA, [3]Nanobiology Institute, Yale University, West Haven, CT, USA, [4]Molecular Biophysics and Biochemistry, Yale University, New Haven, CT, USA, [5]Chemical Engineering, Columbia University, New York, NY, USA.

Fusion of viral and host membranes is a key step during infection by membrane-enclosed viruses. The fusion pore plays a critical role, and must dilate to release the viral genome. Previous studies of fusion mediated by influenza A hemagglutinin (HA) revealed ~2-5 nm pores that flickered before dilating to >10 nm. The mechanisms are unknown.

Here we studied HA-mediated fusion pore dynamics using a novel single-pore assay, combined with computational simulations accessing extraordinarily long ms-s timescales. We measured pores between HA-expressing fibroblasts and bilayer nanodiscs. From pore currents we infer pore size with millisecond time resolution. Unlike previous *in vitro* studies, use of nanodiscs limited the membrane contact areas and maximum pore sizes, better mimicking the initial phases of virus-endosome fusion. With wild type (WT) HA, fusion pores flickered about a mean pore size ~1 nm. By contrast, fusion pores formed by GPI-anchored HA nucleated at half the WT rate and were significantly larger. We developed radically coarse-grained, explicit lipid molecular dynamics simulations of the fusion pore reconstituted with post-fusion, *trans* HA hairpins. With WT HA, fusion pores were small, similar to experiment. Over time hairpins gradually converted from *trans* to *cis*, but contrary to a common view, *cis* hairpins accumulated on the ''viral'' membrane, not the pore waist, due to the low mobility HA transmembrane domains. With GPI-HA the anchoring lipids were far more mobile and the *trans-cis* transition much accelerated. Once most hairpins had converted to *cis*, because apposing membranes were released the fusion pore dilated significantly.

Our results suggest pore dilation requires the *trans-cis* transition. We hypothesize that this transition is accelerated in GPI-HA by the more mobile lipid anchor, explaining the larger observed pores.

409-Pos Board B174

The Influence of Membrane Composition on the Kinetics of Influenza Virus Fusion Measured using a Single Particle Approach

Guus van der Borg[1], Scarlett Braddock[1], Jelle S. Blijleven[1], Antoine M. van Ooien[2], Wouter H. Roos[1].
[1]Molecular Biophysics, Zernike Institute, Groningen, Netherlands, [2]School of Chemistry, Wollongong, Australia.

In order to infect a host cell the influenza virus fuses its envelope with the host cell membrane. This fusion of the viral and cell membranes is mediated by the viral surface protein hemagglutinin (HA) which both docks the virus and fuses the membranes. Using Total Internal Reflection Fluorescence (TIRF) microscopy and a planar, fluid bilayer it is possible to study the kinetics of hemifusion in single virus particles. We have used this technique to study the yield and kinetics of the fusion of influenza with multiple different bilayer compositions. This allows us to better understand the physics behind HA mediated membrane fusion, and thus to better understand the process of influenza infection.

410-Pos Board B175

Revisit the Correlation between the Elastic Mechanics and Fusion of Lipid Membranes

Zih-An Fan, Kuan-Yu Tsang, Si-Han Chen, **Yi-Fan Chen**.
Department of Chemical and Materials Engineering, National Central University, Taoyuan County, Taiwan.

Membrane fusion is a vital process in key cellular events. The fusion capability of a membrane depends on its elastic properties and varies with its lipid composition. It is believed that as the composition varies, the consequent change in C_0 (monolayer spontaneous curvature) is the major factor dictating fusion, owing to the associated variation in G_Es (elastic energies) of the fusion intermediates (e.g. stalk). By exploring the correlations among fusion, C_0 and K_{cp} (monolayer bending modulus), we revisit this long-held belief and re-examine the fusogenic contributions of some relevant factors. We observe that not only C_0 but also K_{cp} variations affect fusion, with depression in K_{cp} leading to suppression in fusion. Variations in G_E and inter-membrane interactions cannot account for the K_{cp}-fusion correlation; fusion is suppressed even as the G_Es decrease with K_{cp}, indicating the presence of factor(s) with fusogenic importance overtaking that of G_E. Furthermore, analyses find that the C_0 influence on fusion is effected via modulating G_E of the pre-fusion planar membrane, rather than stalk. The results support a recent proposition calling for a paradigm shift from the conventional view of fusion and may reshape our understanding to the roles of fusogenic proteins in regulating cellular fusion machineries.

411-Pos Board B176

Hemagglutinin Palmitoylation Contributes to Membrane Curvature in Influenza a Virus Assembly and Membrane Fusion

Petr Chlanda[1], Elena Mekhedov[1], Hang Waters[1], Alexander Sodt[2], Paul S. Blank[1], **Joshua Zimmerberg**[1].
[1]SIB DBTB NICHD, NIH, Bethesda, MD, USA, [2]UMCP DBTB NICHD, NIH, Bethesda, MD, USA.

Three cysteine residues in the cytoplasmic tail of influenza virus glycoprotein hemagglutinin (HA) are covalently modified by three fatty acids and highly conserved among HA subtypes. The importance of these S-acylation post-translational modifications is highlighted by a strain-dependence to their role in virus replication either in assembly or in fusion, but the mechanisms by which the modifications exerts any effect are unknown. We studied the effects of HA acylation on influenza virus-like particle (VLP) morphology, glycoprotein spacing, protein incorporation, HA induced curvature, and membrane fusion using cryo-electron tomography (cET), VLP-cell and cell-cell fusion assays, and molecular dynamics. Acylation has a significant effect on VLP envelope curvature but is not a determinant of either VLP morphology or HA lateral spacing. De-acylated mutant HA is correlated with a flatter envelope curvature of the released particles in the absence of the M1 layer compared to wild type HA. The de-acylated mutant HA failed to incorporate an M1 layer within spherical VLP consistent with altered HA-M1 interactions. In cell-cell fusion assays, fusion pore enlargement was not observed, regardless of which strain of influenza was de-acylated (H2 (A/Japan/305/57), H3 (A/Aichi/2/68), H3 (A/Udorn/72)), suggesting that the role of acylation in membrane fusion is viral strain independent. Fusion without pore enlargement could be partially rescued by the expression of M1 and M2 proteins. The spontaneous curvature of palmitate was calculated by molecular dynamic simulations, and was found to be comparable to curvature values derived from VLP size distributions. Our studies indicate that HA acylation is important for both influenza virus assembly and membrane fusion by controlling membrane curvature and modifying HA's interactions with M1.

412-Pos Board B177

HIV Entry: Receptors Cooperate with Membrane Domain Boundaries to form Entry Sites in Host Cells

Sung-Tae Yang, Volker Kiessling, Lukas K. Tamm.
University of Virginia, Charlottesville, VA, USA.

It has been proposed that lipid rafts of host cell membranes play pivotal roles for cell entry of many enveloped viruses including HIV. However, it remains largely unknown why virions would prefer nanoscopic ordered lipid domains over uniformly fluid membrane regions. Here, we show that HIV does not enter cells from within ordered membrane regions, but rather at the boundaries between raft-like and non-raft-like regions of the plasma membrane. Using cell-derived giant plasma membrane vesicles (GPMVs), which are phase-separated into large-scale liquid-ordered (Lo) and liquid-disordered (Ld) membrane domains, we demonstrate that the HIV receptor CD4 is substantially sequestered into Lo domains while the coreceptor CCR5 localizes preferentially at Lo/Ld domain boundaries. Lo/Ld phase coexistence is not required for HIV attachment, but the recognition of Lo/Ld boundaries is a prerequisite for successful fusion of the viral envelope with the cell membrane. We propose that virions localized to membrane domain boundaries utilize their interfacial energy as an additional driving force for fusion and cell entry. This study provides surprising answers to the long-standing question about the roles of lipid rafts in cell entry of HIV and perhaps other enveloped viruses.

413-Pos Board B178

Single-Virus Observation of pH-Triggered Zika Fusion in the Absence of a Cellular Receptor

Robert J. Rawle[1], Elizabeth Webster[2], Isabel Goronzy[2], Steven Boxer[2], Peter Kasson[1].
[1]Molecular Physiology and Biological Physics, University of Virginia, Charlottesville, VA, USA, [2]Chemistry, Stanford University, Stanford, CA, USA.

Zika virus is a membrane-enveloped flavivirus which has garnered international attention as an emerging pathogen with causal links to birth defects and neurological sequelae following infection. Because Zika virus has only recently been the subject of intense scientific study, little is known about the entry of Zika virus into host cells. From limited studies and by drawing parallels to closely-related flaviviruses, Zika virus is presumed to first bind to a (as yet unknown) receptor on the cell surface, and then become internalized by endocytosis. At some point during the endocytic pathway, the viral E protein is triggered by an unknown factor or factors, initiating membrane fusion with the endosomal membrane. To identify the factor(s) which trigger Zika virus fusion, as well as to study the subsequent fusion kinetics, we used synthetic DNA-lipid conjugates to tether Zika virus to target model lipid membranes in the absence of receptor, a strategy demonstrated earlier for influenza virus (Rawle et al., 2016, *Biophysical Journal*). This enabled us to screen triggering conditions for Zika virus fusion and to monitor the resulting single virus fusion kinetics by quantitative fluorescence microscopy. We demonstrate that low pH, mimicking that inside the endosome, is sufficient to trigger Zika virus fusion (lipid mixing). We also present the pH-dependence of the Zika virus fusion kinetics, as well as implications for a fusion mechanism.

414-Pos Board B179

Molecular Atlas Imaging and Osteoclast Formation: Multiscale Study of Cell-Cell Fusion Mechanisms

Jesse L. Silverberg[1], Pei Ying Ng[2], Roland Baron[2], Peng Yin[1].
[1]Wyss Institute, Harvard University, Boston, MA, USA, [2]Harvard School of Dental Medicine, Harvard University, Boston, MA, USA.

Advanced strategies for synthetic biomaterial design is a critical application of fundamental research in tissue-scale biomechanics. Top-down experiments seek to understand emergent material properties from the organization of individual constituents. While illuminating, bottom-up approaches are a necessary compliment since they provide information hidden by the complex interactions in many-component multi-scale tissues. Thus, reducing complexity and studying minimal interactions allows us to better anticipate and predict phenomenology in biomaterials engineered with living cells. As a concrete example, fracture resistance in bone arises from mineral turnover driven by osteoclast and osteoblast cells. Osteoclasts are particularly interesting since these large multi-nuclear cells resorb bone matrix. While it is widely accepted that efficient bone resorption requires osteoclasts to become multi-nucleated, the exact biological mechanisms initiating and driving cell-to-cell fusion remains poorly understood. In this work, we are using *Molecular Atlas Platform* imaging technology to study the formation of osteoclast cells from the fusion of progenitor bone marrow macrophages. These experiments utilize multiscale imaging to study the cytoskeleton of progenitor cells fusing to create multi-nuclear osteoclasts. At the smallest scales, super-resolution imaging enabled by DNA-PAINT allows us to observe the mechanisms when two progenitor cells come into physical contact and their membranes fuse. At the largest scales, we study osteoclast development over fields of view spanning several mm. By